

IN THE CLAIMS

This listing of claims replaces all prior versions, and listings, in this application.

1. (currently amended) A modified polypeptide having β -glycosidase activity, said polypeptide comprising an amino acid sequence selected from:

- (a) the amino acid sequence of SEQ ID NO:2 mutated ~~[[with]] at least one or more mutations in an amino acid residue or residues~~ selected from the group consisting of W433, E432 and M439 and combinations thereof;
- (b) the amino acid sequence of a family 1 glycosyl hydrolase mutated at an amino acid residue or residues corresponding to ~~at least one~~ an amino acid residue or residues selected from the group consisting of W433, E432 and M439 of SEQ ID NO:2 and combinations thereof; and
- (c) a variant of (a) having β -glycosidase activity and mutated ~~[[with]] at~~ an amino acid residue or residues corresponding to ~~at least one~~ an amino acid residue or residues selected from the group consisting of W433, E432 and M439 of SEQ ID NO:2, wherein said variant has at least 95% identity to SEQ ID NO:2 and combinations thereof over the entire length of the sequence;

~~and said polypeptide having an optional mutation of a catalytic nucleophilic residue of the active site.~~

2. (original) The polypeptide according to claim 1 in which the mutation is selected to broaden the substrate specificity of the polypeptide compared to a polypeptide not so modified.

3. (original) The polypeptide according to claim 1, wherein the mutation is an amino acid substitution.

4. (previously presented) The polypeptide according to claim 1 in which the polypeptide comprises:

- (i) SEQ ID NO:2 having one or more of W433, E 432 and M439 substituted by cysteine, valine or alanine; or
- (ii) the amino acid sequence as defined in (b) or (c) having one or more of the amino acid residues corresponding to W433, E432 and M439 of SEQ ID NO:2 substituted by cysteine, valine or alanine.

5. (currently amended) A modified polypeptide having β -glycosidase activity, said polypeptide comprising an amino acid sequence selected from:

- (a) the amino acid sequence of SEQ ID NO:2 mutated ~~[[with]]~~ at least one or more mutations an amino acid residue or residues selected from the group consisting of ~~[[W433C]]~~ W433, ~~[[E432C]]~~ E432 and ~~[[M439C]]~~ M439 and combinations thereof, wherein each of said mutated amino acid residue(s) is substituted with a cysteine residue;
- (b) the amino acid sequence of a family 1 glycosyl hydrolase mutated in an amino acid residue corresponding to at least one of W433, E432 and M439 of SEQ ID NO:2 and combinations thereof, wherein each of the mutated amino acid recidue(s) is substituted by a cysteine residue; and
- (c) a variant of (a) having β -glycosidase activity and mutated ~~[[with]]~~ at an amino acid residue or residues corresponding to at least one an amino acid residue or residues selected from the group consisting of W433, E432 and M439 of SEQ ID NO:2 and combinations thereof, wherein each of the mutated amino acid residue(s) is substituted by a cysteine residue and wherein said variant has at least 95% identity to SEQ ID NO:2 over the entire length of the sequence.

6. (previously presented) The polypeptide according to claim 5, wherein the cysteine residue introduced by the mutation is chemically modified.

7. (previously presented) The polypeptide according to claim 6, wherein the cysteine residue is modified so as to comprise a positively-charged group.

8. (original) The polypeptide according to claim 7, wherein the positively charged group is of formula $-(CH_2)_n-N^+R_3$, wherein n is a positive integer from 1 to 4 and each R, which may be the same or different, is H or a C₁-C₄ alkyl group.

9. (original) The polypeptide according to claim 8, wherein the positively charged group is $-CH_2CH_2NMe_3^+$.

10. (previously presented) The polypeptide according to claim 6, wherein the cysteine residue is modified so as to comprise a negatively-charged group.

11. (original) The polypeptide according to claim 10, wherein the negatively-charged group is of formula $-(CH_2)_n-SO_3^-$ or $-(CH_2)_n-COO^-$, wherein n is a positive integer from 1 to 4.

12. (original) The polypeptide according to claim 11, wherein the negatively-charged group is of formula $-(CH_2)_n-SO_3^-$.

13. (previously presented) The polypeptide according to claim 6, wherein the cysteine residue is modified so as to comprise an uncharged group.

14. (original) The polypeptide according to claim 13, wherein the uncharged group is a C₁-C₄ alkyl group.

15. (original) The polypeptide according to claim 14, wherein the uncharged group is methyl.

Claims 16-22 (canceled)

23. (original) The polypeptide according to claim 6, wherein the family 1 glycosyl hydrolase is *Sulfolobus solfataricus* β -glycosidase.

Claims 24-26 (canceled)

27. (withdrawn/currently amended) A method for hydrolysing a β -glycoside, which method comprises contacting a glycoside substrate with a modified polypeptide having β -glycosidase activity, said polypeptide comprising an amino acid sequence selected from:

- (a) the amino acid sequence of SEQ ID NO:2 mutated ~~[[with]]~~ at least one or more mutations in an amino acid residue or residues selected from the group consisting of W433, E432 and M439 and combinations thereof;
- (b) the amino acid sequence of a family 1 glycosyl hydrolase mutated ~~[[in]]~~ at an amino acid residue or residues corresponding to at least one an amino acid residue or residues selected from the group consisting of W433, E432 and M439 and combinations thereof of SEQ ID NO:2; and
- (c) a variant of (a) having β -glycosidase activity and mutated ~~[[with]]~~ at an amino acid residue or residues corresponding to at least one an amino acid residue or residues selected from the group consisting of W433, E432 and M439 of SEQ ID NO:2 and combinations thereof, wherein said variant has at least 95% identity to SEQ ID NO:2 over the entire length of the sequence;

~~and said polypeptide having an optional mutation of a catalytic nucleophilic residue of the active site.~~

28. (withdrawn) The method according to claim 27, wherein the glycoside substrate is selected from the group consisting of a glucoside, a galactoside, a fucoside, a xyloside, a mannoside, and a glucuronide.

29. (withdrawn) The method according to claim 27, wherein the polypeptide is contacted with a sample containing at least two different glycosides.

30. (withdrawn/currently amended) A method for hydrolysing a β -glycoside, which method comprises contacting a glycoside substrate with a modified polypeptide having β -glycosidase activity, said polypeptide comprising an amino acid sequence selected from:

- (a) the amino acid sequence of SEQ ID NO:2 mutated ~~[[with]]~~ at least one or more mutations an amino acid residue or residues selected from the group consisting of W433C, E432C and M439C and combinations thereof, wherein each of said mutated amino acid residue(s) is substituted with a cysteine residue;
- (b) the amino acid sequence of a family 1 glycosyl hydrolase mutated ~~[[in]]~~ at an amino acid residue or residues corresponding to ~~at least one~~ an amino acid residue or residues selected from the group consisting of W433, E432 and M439 of SEQ ID NO:2 and combinations thereof, wherein each of the mutated amino acid residue(s) is substituted by a cysteine residue; and
- (c) a variant of (a) having β -glycosidase activity and mutated ~~[[with]]~~ at an amino acid residue or residues corresponding to ~~at least one~~ an amino acid residue or residues selected from the group consisting of W433, E432 and M439 of SEQ ID NO:2 and combinations thereof, wherein each of the mutated amino acid residue(s) is substituted by a cysteine residue, and wherein said variant has at least 95% identity to SEQ ID NO:2 over the entire length of the sequence; wherein the cysteine ~~residue~~ residue(s) introduced by the mutation of (a), (b) or (c) is chemically modified.

31. (withdrawn) The method according to claim 30, wherein the glycoside substrate is selected from the group consisting of a glucoside, a galactoside, a fucoside, a xyloside, a mannoside, and a glucuronide.

32. (withdrawn) The method according to claim 30, wherein the polypeptide is contacted with a sample containing at least two different glycosides.

Claims 33-37 (canceled)

38. (previously presented) The polypeptide according to claim 1, wherein the variant (c) has at least 99% identity to SEQ ID NO:2 over the entire length of the sequence.

39. (currently amended) The polypeptide according to claim 1, said polypeptide comprising the amino acid sequence of a family 1 glycosyl hydrolase mutated ~~[[in]]~~ at an amino acid residue or residues corresponding to ~~at least one~~ an amino acid residue or residues selected from the group consisting of W433, E432 and M439 of SEQ ID NO:2 and combinations thereof.

40. (currently amended) The polypeptide according to claim 39, wherein each of said mutation(s) consists of substitution of the amino acid residue ~~corresponding to at least one of W433, E432 and M439 of SEQ ID NO:2~~ by an amino acid residue selected from the group consisting of cysteine, valine or alanine.

Claim 41 (canceled)

42. (currently amended) The polypeptide according to claim 1 having β -glycosidase activity, and comprising an amino acid sequence having at least 95% identity to SEQ ID NO:2 over the entire length of the sequence and ~~a mutation in~~ mutated at an amino acid residue or residues corresponding to ~~at least one~~ an amino acid residue or residues selected from the group consisting of W433, E432 and M439 of SEQ ID NO:2 and combinations thereof.

43. (currently amended) The polypeptide according to claim 5 having β -glycosidase activity, and comprising an amino acid sequence having at least 95% identity to SEQ ID NO:2 over the entire length of the sequence and ~~a mutation in~~ mutated at an amino acid residue or residues corresponding to ~~at least one~~ an amino acid residue or residues selected from the group consisting of W433, E432 and M439 of SEQ ID NO:2 and

combinations thereof, wherein each of the mutated amino acid residue(s) is substituted by a cysteine residue.

44. (withdrawn/currently amended) The method according to claim 27, wherein said polypeptide has β -glycosidase activity, and ~~comprising wherein said polypeptide~~ comprises an amino acid sequence having at least 95% identity to SEQ ID NO:2 over the entire length of the sequence and ~~a mutation in~~ mutated at an amino acid residue or residues corresponding to ~~at least one~~ an amino acid residue or residues selected from the group consisting of W433, E432 and M439 of SEQ ID NO:2 and combinations thereof.

45. (withdrawn/currently amended) The method according to claim 30, wherein said polypeptide has β -glycosidase activity, and ~~comprising wherein said polypeptide~~ comprises an amino acid sequence having at least 95% identity to SEQ ID NO:2 over the entire length of the sequence and ~~a mutation in~~ mutated at an amino acid residue or residues corresponding to ~~at least one~~ an amino acid residue or residues selected from the group consisting of W433, E432 and M439 of SEQ ID NO:2 and combinations thereof, wherein each of the mutated amino acid residue(s) is substituted by a cysteine residue.